Zika Virus Detailed Laboratory Ordering Guidance

For testing infants and specimen collection at delivery – go to Detailed Testing Guidance by Patient Type #5

Serum and urine are the primary diagnostic specimens for Zika virus infection. For all diagnostic testing conducted on specimen types other than serum, it is also necessary to concurrently obtain a serum specimen for reflex IgM testing.

http://www.doh.wa.gov/ForPublicHealthandHealthcareProviders/PublicHealthLaboratories/MicrobiologyLabTestMenu

Detailed Testing Guidance by Patient Type

(Pregnant, asymptomatic, symptomatic, infant)

1. I've reviewed the Zika virus testing criteria and I have a symptomatic non-pregnant patient with possible Zika virus exposure. What now?

All symptomatic patients with travel to an area where dengue and/or chikungunya may also be circulating should be tested for these viruses at a commercial laboratory

- **<14 days since symptom onset:**
  Obtain serum and urine (whole blood can also be collected), order molecular testing (RT-PCR). RT-PCR is available at CDC or commercial labs. RT-PCR positive is confirmation of infection but RT-PCR negative does not rule out infection. If RT-PCR is negative, antibody testing is required. IgM antibody testing is available at CDC or commercial labs. If IgM antibody tests are negative, recent Zika virus infection is ruled out. If IgM antibody tests are positive or equivocal for Zika or dengue, PRNT testing is required. PRNT tests are only available at CDC.
- **≥14 days – 12 weeks since symptom onset:** Obtain serum only, order IgM antibody testing. IgM antibody testing is available at CDC or commercial labs. If IgM antibody tests are negative, recent Zika virus infection is ruled out. If IgM antibody tests are positive or equivocal for Zika or dengue, PRNT testing is required. PRNT tests are only available at CDC.

2. I've reviewed the Zika virus testing criteria and I have a symptomatic pregnant patient with possible Zika virus exposure. What now?

All symptomatic patients with travel to an area where dengue and/or chikungunya may also be circulating should be tested for these viruses at a commercial laboratory

- **<14 days since symptom onset:** Obtain serum and urine (whole blood can also be collected), order molecular testing (RT-PCR). RT-PCR is available at CDC or commercial labs. RT-PCR positive is confirmation of infection but RT-PCR negative does not rule out infection. If RT-PCR is negative, antibody testing is required. IgM antibody testing is available at CDC or commercial labs. If IgM antibody tests are negative, recent Zika virus infection is ruled out. If IgM antibody tests are positive or equivocal for Zika or dengue, PRNT testing is required. PRNT tests are only available at CDC.
- **≥14 days – 12 weeks since symptom onset:** Obtain serum and urine (whole blood can also be collected), order IgM antibody testing on serum. IgM antibody testing is available at CDC or commercial labs. If IgM antibody tests are negative, recent Zika virus infection is ruled out. If IgM antibody tests are positive or equivocal, test available specimens by RT-PCR. RT-PCR positive is confirmation of infection. If RT-PCR tests are negative, PRNT testing is required. PRNT tests are only available at CDC. If PRNT testing is inconclusive, (e.g. unspecified flavivirus), **additional specimens*** should be collected at delivery for further testing.
- **>12 weeks since symptom onset – delivery:** Testing can be considered but results from specimens obtained >12 weeks after symptom onset may not be definitive (e.g. a negative IgM does not rule out infection) and additional testing at the time of delivery might be indicated. If testing, obtain serum and urine (whole blood can also be collected), order IgM antibody testing on serum. IgM antibody testing is available at CDC or commercial labs. A negative IgM result does not rule out infection, and **additional specimens*** can be collected at delivery for further testing. If IgM antibody tests are positive or equivocal, test available specimens by RT-PCR. RT-PCR positive is confirmation of infection. If RT-PCR tests are negative, PRNT testing is required. PRNT tests are only available at CDC. If PRNT testing is inconclusive, (e.g. unspecified flavivirus), **additional specimens*** should be collected at delivery for further testing. Serial ultrasounds should be considered.

3. I've reviewed the Zika virus testing criteria and I have an asymptomatic pregnant patient with possible Zika virus exposure. What now?

- **<14 days since possible exposure:** Obtain serum and urine (whole blood can also be collected), order molecular testing (RT-PCR). RT-PCR is available at CDC or commercial labs. RT-PCR positive is confirmation of infection but RT-PCR negative does not rule out infection. If RT-PCR is negative, collect a serum specimen 2-12 weeks after last possible exposure for antibody testing.
- **≥14 days – 12 weeks since possible exposure:** Obtain serum and urine (whole blood can also be collected), order IgM antibody testing on serum. IgM antibody testing is available at CDC or commercial labs. If IgM antibody tests are negative, recent Zika virus infection is ruled out. If IgM antibody tests are positive or equivocal for Zika or dengue, test available specimens by RT-PCR. RT-PCR positive is confirmation of infection but RT-PCR negative does not rule out infection. If RT-PCR tests are negative, PRNT

testing is required. PRNT tests are only available at CDC. If PRNT testing is inconclusive, (e.g. unspecified flavivirus), **additional specimens*** should be collected at delivery for further testing.

- **>12 weeks since possible exposure – delivery:** Testing can be considered but results from specimens obtained >12 weeks after symptom onset may not be definitive (e.g. a negative IgM does not rule out infection) and additional testing at the time of delivery might be indicated. If testing, obtain serum and urine (whole blood can also be collected), order IgM antibody testing on serum. IgM antibody testing is available at CDC or commercial labs. A negative IgM result does not rule out infection, and **additional specimens*** can be collected at delivery for further testing. If IgM antibody tests are positive or equivocal for Zika or dengue, test available specimens by RT-PCR. RT-PCR positive is confirmation of infection. If RT-PCR tests are negative, PRNT testing is required. PRNT tests are only available at CDC. If PRNT testing is inconclusive, (e.g. unspecified flavivirus), **additional specimens*** should be collected at delivery for further testing. Serial ultrasounds should be considered.

4. I've reviewed the Zika virus testing criteria and I have a pregnant patient with possible Zika virus exposure and fetal abnormalities present on ultrasound. What now?

- If fetal abnormalities are present on ultrasound, testing should be performed. Women who originally tested negative or who were not tested for Zika virus infection following exposure should be tested/retested for Zika virus infection. Zika virus RT-PCR can be performed on serum, urine, whole blood, and amniotic fluid. The sensitivity and specificity of this test are currently unknown for congenital infection. It is also unknown if a positive result is predictive of a subsequent fetal abnormality.

5. I've reviewed the Zika virus testing criteria and I have an infant with laboratory evidence of maternal Zika virus infection, OR

An infant with abnormal clinical or neuroimaging findings suggestive of congenital Zika syndrome AND a maternal epi link, OR

An infant with fever, rash, arthralgia, or conjunctivitis within 2 weeks of delivery and maternal possible exposure* occurred within 2 weeks of delivery:

- **Within 2 days of birth**, collect infant serum and urine for RT-PCR and serologic testing. Whole blood can also be collected. Collection of cord blood is not recommended. CSF obtained for other studies can also be tested. If RT-PCR tests are negative and the infant's initial sample is IgM-positive, but PRNT was not performed on the mother's sample, PRNT should be performed on the infant's initial sample. However, PRNT cannot distinguish between maternal and infant antibodies.

- For circumstances in which maternal testing was not previously performed, was performed more than 12 weeks after exposure, or was not definitive (e.g. unspecified flavivirus), **additional specimens*** can be collected for further testing. If an infant appears clinically well and maternal results are not yet available, Zika virus testing can be deferred until maternal test results are available.

***Additional specimens collected at delivery =**

- Several full thickness pieces of placenta, including at least 3 full thickness pieces (0.5–1 cm x 3–4 cm in depth) from middle third of placental disk and at least 1 from the placental disk margin, should be submitted if available. Include sections of the placental disk, 5x12 cm strip of fetal membranes, and pathologic lesions when possible. Please include information about placenta weight and sample both maternal and fetal sides of the placenta and label all specimens to identify location of sample.
- Umbilical cord segments should be obtained proximal, middle, and distal to umbilical cord insertion site on the placenta. Four or more, 2.5 cm segments of umbilical cord should be submitted, if available. All specimens should be fixed in formalin (volume of formalin about 10x mass of tissue) and held at ambient temperature.

6. I've reviewed the testing criteria and I have a pregnant patient experiencing fetal loss, still birth, a terminated pregnancy, or death of an infant shortly after birth in a woman with laboratory evidence of maternal Zika virus infection, OR with abnormal findings suggestive of congenital Zika syndrome AND a maternal epi link:

The following specimens should be collected for RT-PCR if possible and fixed in formalin:

- Brain tissue (most important to evaluate for possible Zika virus infection), and spinal cord, 5 or more specimens from different parts of the brain and spinal cord, 0.5-1 cm^3 each. Maintain tissue architecture to evaluate viral pathology. Please fix brain specimens 48-72 hrs.
- Placenta should be sampled extensively or submitted intact if early in gestation. Include at least 3 full thickness pieces (0.5-1 cm x 3-4 cm), from the middle third of the placental disk and at least 1 from the placental disk margin. Also include one 5 x 12 cm strip of fetal membranes
- Four or more umbilical cord specimens should be submitted, in 2.5 cm segments.
- If individual organs or tissue types can be easily identified at autopsy, collect 1 representative 0.5-1.0cm^3 sample from: heart, lungs, liver, kidneys, skeletal muscle, eyes and bone marrow. Sampling of eyes is highly recommended.
- For situations where individual organs or tissue types cannot be identified, provide any available tissue with minimal disruption (4 or more specimens if possible).

For more information, go to Zika Virus for Healthcare Providers and Clinical Labs (link).